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Characterization of a *Thelypteris* Hybrid from Walker County, Texas

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Abstract.—A putative hybrid of unknown parentage was discovered in Walker County, Texas. Taxonomic features resemble both *Thelypteris kunthii* and *Thelypteris ovata* var. *lindheimeri* indicating hybridity. Spores of the putative hybrid examined by microscopy appeared malformed and were sterile with 0% germination. Guard cell measurements were intermediate to the presumed diploid and tetraploid parental species suggesting that the hybrid is triploid. Qualitative and quantitative phenetic characters examined were an array of parental and intermediate characters, suggesting that it had resulted from a cross between *Thelypteris kunthii* and *Thelypteris ovata* var. *lindheimeri*.

Ferns have extensive hybridization (Wagner, 1969) and Knobloch (1976) listed 620 fern hybrids, including some alloploids of known parentage. Fertile and sterile hybrids of *Thelypteris* have been described, primarily from Florida and Mexico (Mickel *et al.*, 1966). Smith (1993) described hybrids of *Thelypteris* in Florida between *Thelypteris kunthii* (Desv.) C. V. Morton and *Thelypteris ovata* R. P. St. John var. *ovata*, and between *T. kunthii* and *Thelypteris augescens* (Link) Munz *et* I. M. Johnson.

A *Thelypteris* was discovered on the grounds of the Sam Houston Memorial Museum (SHMM) in Walker County, Texas. The parentage of this taxon is not known. Alan R. Smith (personal communication) suggested it was a hybrid, and that the parents could be *Thelypteris kunthii* and *Thelypteris ovata* var. *lindheimeri* (C. Christensen). Native ranges of the proposed parents do not overlap (Smith, 1993). *Thelypteris kunthii* is native to the southeastern U.S., with its range extending into east Texas and Walker County. *Thelypteris ovata* var. *lindheimeri* is native to north central Texas and west into the Edwards Plateau and Trans-Pecos areas (Smith, 1993; Diggs *et al.*, 1999). Although *T. ovata* var. *lindheimeri* is not native to Walker County, it is grown as an ornamental within three city blocks from the apparent hybrid on the Sam Houston State University (SHSU) campus. The goal of this study was to characterize and determine parentage of the suspected hybrid by comparing phenetic traits with *T. kunthii* and *T. ovata* var. *lindheimeri*.

Materials and Methods

All spores, croziers, and fully developed fronds were collected in Walker County, Texas, from May 1999 until August 2001. Putative hybrid material was collected on the grounds of the SHMM. *Thelypteris ovata* var. *lindheimeri* was collected from a cultivated population on campus at SHSU. *Thelypteris kunthii* was collected from a wild population growing at the SHSU Center for

Biological Field Studies, within ten miles from campus. All spores were stored at 4°C. Herbarium specimens from all three ferns were deposited in the SHSU S. R. Warner Herbarium.

Walter M. Woodward, Curator of Collections, and Dr. Patrick Nolan, Director, of the SHMM were interviewed for a historical background of the hybrid location. Manuscripts written by Martinus H. Stougaard, the first Landscape Architect of the SHMM were consulted at the Peabody Library, SHSU. Grounds keeping and maintenance personnel were interviewed for historical records that would indicate the date *T. ovata* var. *lindheimeri* was planted on campus as an ornamental. The area surrounding the SHMM, two city blocks north, east, south, and west, was surveyed for *Thelypteris* species. Specimens were collected and examined morphologically.

Traits distinguishing the putative parents (Smith, 1971, 1993) were examined and compared with those of the hybrid. Observations and measurements were made from nine specimens of *T. ovata* var. *lindheimeri*, seven specimens of the putative hybrid, and eleven specimens of *T. kunthii*.

We assessed the following morphological characters for evidence of intermediacy in the putative hybrid: 1) overall leaf shape including blade apex; 2) presence or absence of scales on the rachis; 3) pinnae shape and attachment of middle pinnae to the rachis; 4) location of the sori; and 5) presence or absence of stalked sporangial glands. Also, spores were attached to SEM stubs with double sided tape, sputter coated to 200 Å for two minutes with gold palladium, examined, and photographed under a Scanning Electron Microscope (JEOL model JSM 6400 Scanning Microscope) at the Texas A&M University Microscopy Center, College Station, Texas. Spores were mounted in Hoyer's medium on glass slides for measurements of spore length that included the perispore. Photomicrographs were obtained after several weeks to insure maximum swelling had occurred in the spores (Barrington *et al.*, 1986).

We examined light and SEM micrographs of spores for signs of spore malformation. To test spore viability, spores from each fern were surface-sterilized and sown (Nester and Schedlbauer, 1981) under aseptic conditions (Schedlbauer, 1976) onto 1% agar solidified plates containing Parker's macroelements and Thompson's microelements (Klekowski, 1969). Germinated and ungerminated spores in three fields of view per plate were counted. We observed at least 200 spores from each of the three ferns and obtained germination percentages.

Ploidy of the hybrid was determined by statistically comparing the guard cell length of the putative hybrid and the presumed parents. Statistical analyses were performed with Minitab Student Release 12 (Minitab Inc., 1998). Analysis of variance (ANOVA) was used to determine significant differences between the putative parents and the hybrid, and results were considered statistically significant at $\alpha=0.05$. We obtained guard cell lengths from the pinnatifid apex of the leaf, where there were no sori or major veins to interfere with measurements. One to two millimeter leaf pieces were rinsed in 100% ethanol and mounted in Hoyer's medium onto glass slides. Guard cell length was measured using an ocular micrometer and photographed.

Results

Information provided by local historians indicates the putative hybrid most likely appeared near the SHMM some time in the 1930's. Martinus H. Stougaard was employed at the SHMM from 1928 to 1936 as a landscape architect. Manuscripts written by Stougaard do not indicate his participation in the synthesis of this specimen. Unfortunately, we found no direct evidence for synthetic or naturally occurring hybridization of *T. kunthii* and *T. ovata* var. *lindheimeri*. Historical records were not available to validate when *T. ovata* var. *lindheimeri* was planted as an ornamental on campus.

In the past, people in this area could have used *Thelypteris* as an ornamental. Therefore, a two city block radius surrounding the SHMM was surveyed for *Thelypteris*. *Thelypteris* was found growing in six locations. The presence of stalked sporangial glands, sori location, and guard cell size indicated three of the specimens were *T. kunthii*. The presence of stalked sporangial glands, dark hairy scales on the rachis, elongate basal segments of the middle pinnae that are parallel to the rachis, and guard cell size suggested one specimen was the hybrid in question. Persistent tan glabrous scales, middle pinnae with basal segments that are elongate and parallel to the rachis, and guard cell size indicated two specimens were *T. ovata* var. *lindheimeri*.

Characters of the three *Thelypteris* taxa are described below and shown in Table 1. Blade shape for *T. ovata* var. *lindheimeri* was ovate-lanceate. Blade shape varied from ovate-lanceate, lanceolate, to triangular for the hybrid, and was lanceolate, lanceate, to triangular in *T. kunthii*. Both *T. ovata* var. *lindheimeri* and the hybrid had a gradually to somewhat abruptly tapered apex while *T. kunthii* had a gradually tapered apex.

The basal segments of the middle pinnae were elongate and parallel to the rachis in both *T. ovata* var. *lindheimeri* and the hybrid, but not elongate and somewhat oblique to the rachis in *T. kunthii*. Pinna segments in *T. ovata* var. *lindheimeri* were oblique and curved with submarginal sori. Pinna segments in the hybrid were oblique to oblong with rounded to acute apices and supramedial to medial sori. Pinna segments were oblong with rounded to acute apices with supramedial to medial sori in *T. kunthii*.

Scales were dense on the rachis of *T. ovata* var. *lindheimeri*, sparse to dense on the rachis of the hybrid, and sparse on the rachis of *T. kunthii*. Scales on *T. ovata* var. *lindheimeri* were tannish brown, and glabrous to minutely pubescent. Scales of the hybrid and *T. kunthii* were dark brown and pubescent. Yellowish-stalked sporangial glands were present in *T. kunthii* and the hybrid, and absent in *T. ovata* var. *lindheimeri*.

SEM photographs were used to compare spore ornamentation and verify any differences and/or similarities between the hybrid and the proposed parents (Fig. 1). Spores of *T. ovata* var. *lindheimeri* were cristate with continuous wide flat crests, sparsely verrucose, with small pits (Fig. 1a). Spores of *T. kunthii* (Fig. 1b) were cristate with discontinuous thin crests, verrucose, with small pits. The hybrid spores (Fig. 1c) appeared to be cristate, with thin continuous crests, verrucose to tuberculate. Micrographs reveal hybrid spores collapsed

TABLE 1. Morphological characteristics of *T. ovata* var. *lindheimeri*, putative hybrid, and *T. kunthii*.

Character	*T. ovata* var. *lindheimeri*	putative hybrid	*T. kunthii*
Blade shape	Ovate-lanceate	Lanceolate, triangular or ovate-lanceate	Lanceolate, lanceate, or triangular
Apex	Gradually-somewhat abruptly tapered	Gradually-somewhat abruptly tapered	Gradually tapered
Basal pair middle pinnae	Elongate and parallel to rachis	Elongate and parallel to rachis	Not elongate, somewhat oblique to the rachis
Pinnae segments	Oblique to curved	Oblong, oblique to Curved	Oblong
Pinnae apex	Acute	Rounded to acute	Rounded to acute
Sorus location	Submarginal	Supramedial-medial	Supramedial-medial
Rachis Scales	Dense	Sparse-dense	Sparse
Rachis Color	Tannish brown	Dark brown	Dark brown
Rachis hair	Minutely pubescent	Pubescent	Pubescent
Sporangial Glands	Absent	Present	Present
Guard cell Length	32 ± 5 µm	36 ± 4 µm	40 ± 4 µm
Spore length (including perispore)	50 ± 4 µm	46 ± 6 µm	52 ± 7 µm

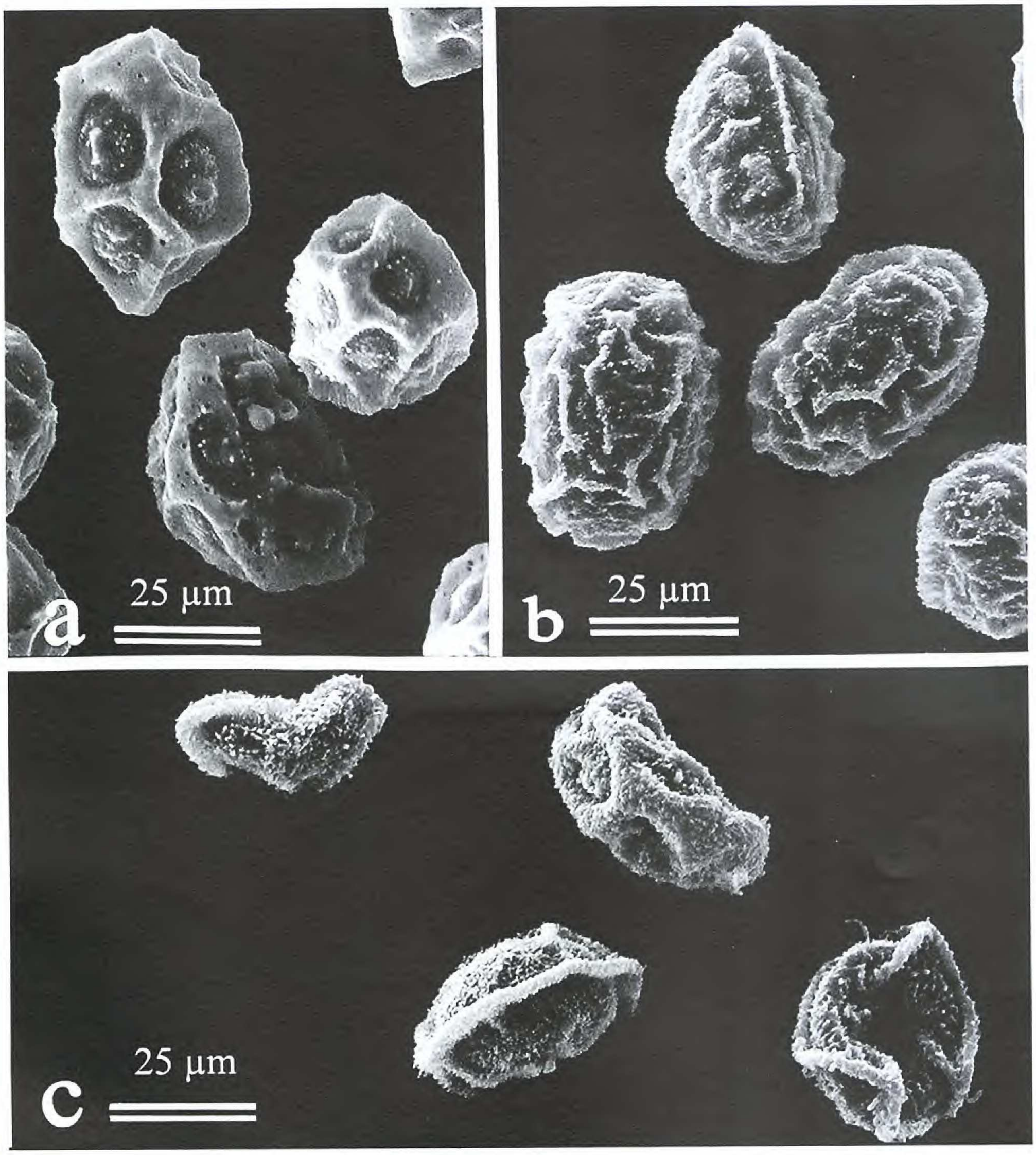

FIG. 1. SEM of spores of *Thelypteris* species and putative hybrid. *Thelypteris ovata* var. *lindheimeri* spores (a) with continuous wide flat crests and small pits, *Thelypteris kunthii* spores (b) with discontinuous thin crests, verrucose with small pits, and hybrid spores (c) with thin continuous crests, verrucose to tuberculate.

and twisted, showing concave walls and strong curved outlines, which are all characteristics of nonviable spores in hybrids. By day 18, germination rate was 65% for *T. kunthii*, 55% for *T. ovata* var. *lindheimeri*, and 0% for the hybrid.

Guard cell length for all three ferns was measured and compared for indications of ploidy level of the hybrid (Table 1). Average guard cell length

was 32 ± 5 μm for *T. ovata* var. *lindheimeri*, 36 ± 4 μm for the putative hybrid, and 40 ± 4 μm for *T. kunthii*. ANOVA showed that the three taxa were significantly different ($P<0.001$).

Spore length (including the perispore) for all three ferns was measured and compared for indications of ploidal level of the hybrid (Table 1). *T. ovata* var. *lindheimeri* had an average spore length of 50 ± 4 μm, the hybrid 46 ± 6 μm, and 52 ± 7 μm for *T. kunthii*.

Discussion

Morphological characters suggest the putative *Thelypteris* hybrid arose from a cross between *T. kunthii* and *T. ovata* var. *lindheimeri*. The place, time and circumstances of this origin are uncertain.

Thelypteris was found in six locations inside the area surveyed. From a comparison of taxonomic characters and guard cell size, we conclude that one of the six specimens collected was a hybrid, three were *T. kunthii*, and the remaining two were *T. ovata* var. *lindheimeri*. *Thelypteris* species appear to be commonly used as ornamentals in the area surveyed and are easily propagated by rhizome fragmentation.

All examined morphological characteristics of the hybrid show a combination of the features of both presumed parents. A common misconception is that hybrids are typically morphologically intermediate between their parents (Reiseberg, 1995). Reiseberg and Ellstrand (1993) found that hybrids are a mosaic of parental, intermediate, and extreme characters. Hybrids commonly express morphological intermediacy, but the characters that are governed by just one or a few genes can have parental, novel, or extreme character states (Reiseberg, 1995).

All viable spores of *T. kunthii and T. ovata* var. *lindheimeri* had germinated by day 18 of the viability test. Spores of the hybrid are malformed, a characteristic of spores in other hybrid pteridophytes (Smith, 1971; Wagner *et al.*, 1986; Brunton and Taylor, 1990; Hoshizaki, 2001), and had 0% germination, which is typical of fern hybrids with uneven chromosome numbers. Malformed spores and their nonviability are strong evidence of hybridization in homosporous pteridophytes. Although spore malformation may sometimes be due to factors other than hybridity, this combination of circumstances has occurred so commonly in ferns that the likelihood of any other explanation is exceedingly small (Wagner et al. 1986).

Cell size has been found to correlate with ploidal level within some fern groups (genera) (Lawton, 1932; Wagner, 1954; Barrington *et al.*, 1986; Rasbach *et al.*, 1994). Results from the statistical analysis of guard cell size measurements were significantly different between the putative diploid and tetraploid parents, and support a hypothesis of hybridization for the plant in question. We further conclude that the plants in question results from hybridization between *T. kunthii* and *T. ovata* var *lindheimerii*.

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